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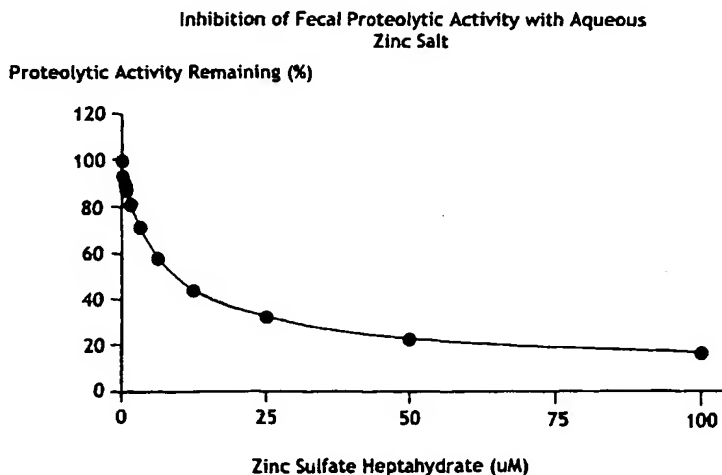
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(54) Title: DELIVERY OF A SKIN HEALTH BENEFIT AGENT TO A TREATED SUBSTRATE FOR TRANSFER TO SKIN



(57) Abstract: The present invention relates to a combination for surface treatment of a substrate, e.g. a nonwoven web, used in personal care product applications. The surface treatment combination not only provides adequate fluid handling properties, but also provides a topical delivery system effective in depositing a thin, tenacious and substantially continuous coating of a skin health benefit agent on skin by an aqueous emulsion mediated dissolution of the agent from a substrate with subsequent transfer and deposition onto the skin. Coatings of the skin health benefit agent on the skin resist removal, thereby preventing damage to the natural skin barrier and providing a protective barrier against chemically- and biochemically-induced skin damage.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

**DELIVERY OF A SKIN HEALTH BENEFIT AGENT TO A
TREATED SUBSTRATE FOR TRANSFER TO SKIN**

FIELD OF THE INVENTION

This invention relates to the use of a skin health benefit agent to enhance skin
5 health. A treatment combination includes a surfactant and skin health benefit agent that
can be applied to a substrate such as a nonwoven web, such that the composition will
impart adequate fluid handling properties to the substrate and will subsequently be
transferred to the skin for enhancing skin health. The treatment composition may further
be used as a vehicle to deliver other agents to the skin, e.g. proteins.

10 **BACKGROUND OF THE INVENTION**

The skin is naturally an excellent barrier to the penetration of many foreign
substances. From time-to-time, the natural ability of the skin to protect is compromised by
external factors including abrasions, irritants and the like. Attempts have been made in
recent years to promote skin health through the use of various products containing
15 additives or developing synthetic or naturally occurring polymers that mimic or
complement the properties of skin in order to maintain the skin health.

It is known that various agents have skin enhancing properties when applied to
skin and hair. Effective delivery of skin health benefit agents that can enhance or prevent
damage to the underlying protective barrier of skin, is not yet known.

20 Enhancing skin health and delivering agents to the skin to promote skin health has
many advantages including: 1) protecting the skin and maintaining the skin in a moist
state, essentially free from chapping or irritation, 2) pH buffering and barrier enhancement
to maintain or enhance such base properties of skin, 3) inhibition of irritants that are
suspected to promote irritant or allergic contact dermatitis, and 4) maintaining the lubricity
25 of skin.

The permeability of the skin to a foreign substance is influenced by a combination of physico-chemical parameters for both the object and the vehicle, if applicable, that delivers the object. Maintaining health of the skin and its underlying barrier properties requires optimal physico-chemical properties of the skin.

5 U.S. Patent No. 4,556,560 to Buckingham, assigned to The Procter & Gamble Company, discloses methods for treatment and prophylaxis of diaper rash and dermatitis using a lipase-inhibiting agent in the form of a water-soluble metallic salt, such as ZnCl_2 .

There is a need for a treatment composition for use with a substrate that is capable of delivering a thin, tenacious, substantially continuous film of the skin health
10 benefit agent to the skin that can prevent or reduce skin irritation, maintain pH, and maintain skin hydration and lubrication. The combination of the instant invention fulfills this need. Additionally, while skin wellness additives are known, other compositions have had the undesired side effect of reducing wettability, or the fluid intake rate, of the substrate. There remains a need for a treatment combination for application and use with
15 a substrate that will not adversely affect fluid handling properties of the substrate, e.g. fast and sustainable fluid intake rate.

SUMMARY OF THE INVENTION

The present invention relates to a combination for surface treatment of a
20 substrate, e.g. a nonwoven web, used in personal care product applications. The surface treatment combination not only provides adequate fluid handling properties, but also provides a topical delivery system effective in depositing a thin, tenacious and substantially continuous coating of a skin health benefit agent on skin by an aqueous emulsion mediated dissolution of the agent from a substrate with subsequent transfer and
25 deposition onto the skin. Coatings of the skin health benefit agent on the skin resist removal, thereby preventing damage to the natural skin barrier and providing a protective barrier against mechanically-, chemically-, and biochemically-induced skin damage. Also

provided is a treatment combination for application and use with a substrate that will not adversely affect fluid properties of the substrate, e.g. fast and sustainable fluid intake rate as long as the material/product is being used.

BRIEF DESCRIPTION OF THE DRAWINGS

5 The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawings will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

FIG. 1 is a schematic illustration of a treating process useful for application of the treatment combination of the present invention to one or both sides of the nonwoven web.

10 FIG. 2 is a partially cut-away top plan view of an exemplary personal care product, in this case a diaper, which may utilize the treated substrate according to the present invention.

FIG. 3 is a 200X optical microscopy (Brightfield and Fluorescent Image) of a substrate treated with a treatment composition according to an aspect of the present
15 invention.

FIG. 4 is a 200X optical microscopy (Fluorescent Image) showing the liquid mediated transfer of the treatment composition from the treated substrate and dissolving in the liquid.

FIG. 5 is a 200X optical microscopy (Fluorescent Image) of skin that has been
20 treated with a treatment composition including liquid mediated transfer of the composition to the skin, wherein the skin health benefit agent has been labeled with a fluorescent dye to show the transfer of a thin, tenacious, substantially continuous film to the skin.

FIG. 6 is a 100X optical microscopy (Brightfield and Fluorescent Image) of skin that has been treated in accordance with the prior art in that the treatment composition
25 has been transferred to the skin by mechanical transfer, wherein a silk protein has been labeled with a fluorescent dye to show the transfer of a substantially discontinuous film to the skin.

FIG. 7 is a graph comparing inhibition of proteolytic activity by zinc sulfate heptahydrate in accordance with the present invention.

FIG. 8 is another graph comparing inhibition of proteolytic activity by zinc sulfate heptahydrate in accordance with the present invention.

5 FIG. 9 is a graph showing reduction of released interleukin-1 alpha by zinc in accordance with the present invention.

FIG. 10 is a graph showing reduction of released interleukin-1 alpha by zinc in accordance with the present invention.

DETAILED DESCRIPTION OF THE INVENTION

10 Combinations and methods are provided by the present invention for topical administration of treatment compositions to the skin of mammals, especially humans, to protect the skin by preserving and restoring the natural integrity of the skin. This is achieved by depositing a skin health benefit agent from a substrate that is able to control the release of the agent to the surface of the skin and that is able to provide adequate

15 handling of body fluids. The skin health benefit agent acts as a protectorant that is capable of maintaining the pH of the skin, inhibit the activity of irritants to the skin, and maintain skin hydration and lubrication. Pancreatic digestive enzymes that are expelled by the body with feces have been implicated to induce skin inflammation (Anderson, P.H., Bucher, A.P., Saees, II, Lee, P.C., Davis, J.A., and Maibach, H.I., Faecal enzymes: in vivo

20 skin irritation. *Contact Dermatitis* 1994; 30, 152-158). When the feces, including these enzymes, contact the skin, the skin becomes irritated. In some cases, proteases, in feces and urine cleave stratum corneum proteins that can cause the breakdown of the natural protective barrier of the skin. The skin is now susceptible to becoming irritated by these molecules as well as other components in the feces and urine. The treatment

25 composition of the present invention is designed to form a thin, tenacious, substantially continuous film over the skin to inhibit, or at least minimize, the effect of such irritants.

The treatment composition of the present invention includes a surfactant system and a skin health benefit agent. Preferably, the treatment composition is prepared as an emulsion of the surfactant and skin health benefit agent, usually as an oil-in-water (o/w) emulsion.

5 Examples of emulsions include aqueous emulsions of skin health benefit agent, e.g. zinc sulfate heptahydrate, and surfactant, e.g. AHCOVEL Base N-62 (hereinafter "AHCOVEL"), a mixture of sorbitan monooleate and polyethoxylated hydrogenated castor oil, manufactured by Hodgson Co. It has been found that when emulsions containing about 75 wt.% surfactant and up to about 25 wt.% skin health benefit agent at about 0.1
10 to 40 wt.% total solids are used, sufficient amounts of the skin health benefit agent transfer to the skin. Preferably, the emulsions will contain between about 5 to 30 wt.% solids. These emulsions can either be applied onto a substrate from a high-solids bath (up to 40 wt.%) or from dilute baths ranging from 0.1 wt.% to about 20 wt.%. Preferably, the emulsion will be diluted to about 0.5 wt.% to about 15 wt.%.

15 The surfactants useful in the treatment composition of the present invention will provide superior fluid handling performance, skin protection and mildness to human skin. Useful examples of the surfactant include ethoxylated fatty acids and alcohols, monosaccharides, monosaccharides derivatives, polysaccharides, polysaccharide derivatives, and combinations thereof.

20 Water miscible nonionic surfactants are preferred and such surfactants are commercially available. Examples of such surfactants include AHCOVEL and Glucopon 220UP, available from Henkel Corporation, which is an alkylpolyglycoside having 8 to 10 carbons in the alkyl chain, and may also be used as a part of the surfactant. Other well known nonionic surfactants are the primary aliphatic alcohol ethoxylates, secondary
25 aliphatic alcohol ethoxylates, alkylphenol ethoxylates and ethylene-oxide-propylene oxide condensates on primary alkanols, such as PLURAFACS and PLURONICS (available from BASF, Inc.) and condensates of ethylene oxide with sorbitan fatty acid esters such as the

TWEEN series (also available from Uniqema). The nonionic surfactants generally are the condensation products of an organic aliphatic or alkyl aromatic hydrophobic compound and hydrophilic ethylene oxide group. Practically any hydrophobic compound having a carboxy, hydroxy, amido, or amino group with a free hydrogen attached to the nitrogen

5 can be condensed with ethylene oxide or with the polyhydration product thereof, polyethylene glycol, to form a water miscible nonionic surfactant. Other suitable surfactants include polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan trioleate, polyoxyethylene sorbitan tristearate, and bovine lipid extract surfactant (Survanta, Ross Laboratories), a drug used to treat

10 Acute Respiratory Distress Syndrome and Cystic Fibrosis, and enzymes such as papain or pepsin which cleave protein structures.

More specifically, the nonionic surfactant may include the condensation products of a higher alcohol (e.g., an alkanol containing about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 carbon atoms in a straight or branched chain configuration) condensed with about 5 to

15 30 moles of ethylene oxide. Examples include: lauryl or myristyl alcohol condensed with about 16 moles of ethylene oxide (EO); tridecanol condensed with about 6 moles of EO; myristyl alcohol condensed with about 10 moles of EO per mole of myristyl alcohol; the condensation product of EO with a cut of coconut fatty alcohol containing a mixture of fatty alcohols with alkyl chains varying from 10, 11, 12, 13 or 14 carbon atoms in length

20 and wherein the condensate contains either about 6 moles of EO per mole of total alcohol or about 9 moles of EO per mole of alcohol; and tallow alcohol ethoxylates containing 6 EO to 11 EO per mole of alcohol. Condensates of 2 to 30 moles of ethylene oxide with sorbitan mono- and $3C_{10}$ - C_{20} alkanoic acid esters having a HLB (hydrophilic/lipophilic balance) of about 4 to 20, preferably about 8 to 15, may also be employed as the nonionic

25 surfactant.

Another class of surfactant compounds include the alkyl polysaccharides. Alkyl polysaccharides are alkyl polyglycosides having the formula SUGAR-O-R, where R is a hydrophobic group.

Turning to the skin health benefit agent, the present invention provides zinc
5 compositions to reduce or prevent skin irritation and/or acute inflammatory reactions of the skin. Examples of such agents include zinc salt, zinc sulfate monohydrate, and the like. Advantageously, the zinc salt will be present in the composition as an aqueous emulsion. These agents are useful as astringents and enzyme inhibitors, and more particularly useful in inhibiting both fecal and urine proteases. Zinc can either directly
10 interact with the catalytic site of the protease, in particular, the class of proteases known as serine proteases, to inhibit proteolytic activity. By inhibiting the proteolytic activity, the intent is to keep the skin from ever becoming irritated, rather than treating the skin once it has become irritated. A further advantage of the skin health benefit agent of the present invention relates to lowering the pH. The serine proteases, e.g. trypsin and pancreatic
15 elastase, which are present in, for example, feces and urine, have a pH optimum of approximately 8.0 and 8.5, respectively. The skin health benefit agent of the present invention has unexpectedly been found to lower the pH, thereby decreasing the catalytic efficiency of these proteases.

The amount of skin health benefit agent will be introduced in the combination
20 described above in the range of from about 0.01% to about 10% by weight of the composition. Preferably, the agent will be present in the amount of about 0.25% to about 1% by weight of the composition.

Compositions and methods are also provided by the present invention for topical administration of the skin health benefit agent concurrently with a protein that can be
25 administered topically in a controlled manner.

One such protein is sericin. Sericin is one of two proteins that are part of the twin fibroin silk thread spun by *Bombyx mori*, a domestic insect. Sericin acts as a protective

envelope around the fibroin thread as it is spun, which is like spinning of fibers with soluble sizing agents to help form good quality fibers. The sericin can be easily separated from silk protein by hydrolysis. Post-spun sericin, with its unique properties, is known to have high affinity to a number of proteins. When refined to a high molecular weight

5 substance it is amenable to binding to the keratin of skin and hair, forming a resistant, moisturizing, and protective film on the skin/hair, imparting good barrier properties.

Sericin is a silk protein obtained by controlled hydrolysis of low molecular weight silk having a specific gravity of at least about 1. A commercially available silk protein is available from Croda, Inc., of Parsippany, NJ, and is sold under the trade name CROSILK

10 LIQUID (silk amino acids), CROSILK 10,000 (hydrolyzed silk), CROSILK POWDER (powdered silk), and CROSILKQUAT (cocodimonium hydroxypropyl silk amino acid). Another example of a commercially available silk protein is SERICIN, available from Pentapharm, LTD, a division of Kordia, bv, of the Netherlands. Further details of such silk protein mixtures can be found in U.S. Patent No. 4,906,460, to Kim, et al., assigned to

15 Sorencor, which is herein incorporated by reference in its entirety.

The silk protein derivatives may be chosen from one of several potential compositions. Included among the silk derivatives are silk fibers and hydrolysate of silk fibers. The silk fibers may be used in the form of powder in preparing the emulsion or as a powder of a product obtained by washing and treating the silk fibers with an acid.

20 Preferably, silk fibers are used as a product obtained by hydrolysis with an acid, alkali or enzyme, as disclosed in U.S. Patent No. 4,839,168 to Abe et al.; U.S. Patent No. 5,009,813 to Watanube et al., and U.S. Patent No. 5,069,898 to Goldberg, each incorporated herein by reference in its entirety.

Another silk derivative that may be employed in the composition of the present

25 invention is protein obtained from degumming raw silk, as disclosed, for example, in U.S. Patent No. 4,839,165 to Hoppe et al., incorporated herein by reference in its entirety. The

principal protein obtained from the raw silk is sericin, which has an empirical formula of $C_{15}H_{25}O_3N_5$ and a molecular weight of about 323.5.

A preferred silk derivative is a mixture of two or more individual amino acids, which naturally occur in silk. The principal silk amino acids are glycine, alanine, serine and
5 tyrosine.

Another example of a silk derivative for use in the emulsion composition of the present invention is a fine powder of silk fibroin in nonfibrous or particulate form, as disclosed in U.S. Patent No. 4,233,212 to Otoi et al., incorporated herein by reference in its entirety.

10 The fine powder is produced by dissolving a degummed silk material in at least one solvent selected from, for example, an aqueous cupriethylene diamine solution, an aqueous ammonia solution of cupric hydroxide, an aqueous alkaline solution of cupric hydroxide and glycerol, an aqueous lithium bromide solution, an aqueous solution of the chloride, nitrate or thiocyanate of calcium, magnesium or zinc and an aqueous sodium
15 thiocyanate solution. The resulting fibroin solution is then dialyzed. The dialyzed aqueous silk fibroin solution, having a silk fibroin concentration of from about 3 to 20% by weight, is subjected to at least one treatment for coagulating and precipitating the silk fibroin, such as, for example, by the addition of a coagulating salt, by aeration, by coagulation at the isoelectric point, by exposure to ultrasonic waves, by agitation at high
20 shear rate and the like.

The resulting product is a silk fibroin gel, which may be incorporated directly into a treatment composition or the same may be dehydrated and dried into a powder and then dissolved in the treatment composition.

The silk material used to form the silk fibroin includes cocoons, raw silk, waste
25 cocoons, raw silk waste, silk fabric waste and the like. The silk material is degummed or freed from sericin by a conventional procedure such as, for example, by washing in warm water containing a surfactant-active agent or an enzyme, and then dried. The degummed

material is dissolved in the solvent and preheated to a temperature of from about 60 to 95°C, preferably of from about 70 to 85°C. Further details of the process of obtaining the silk fibroin are discussed in previously referenced U.S. Patent No. 4,233,212.

In addition to the silk protein of the treatment composition of the present invention,
5 an additional protein may be present in the amount of about 0.1 to about 4.0% by weight. This additional protein may be selected from the group consisting of hydrolyzed animal collagen protein obtained by an enzymatic hydrolysis, lexeine protein, vegetal protein and hydrolyzed wheat protein and mixtures thereof.

The composition of the present invention can be in the form of an oil-in-water (o/w)
10 emulsion or after dilution with water, with the essential ingredients being water, surfactant, and/or co-surfactant.

Because the composition as prepared is an aqueous liquid formulation and since no particular mixing is required to form the o/w emulsion, the composition is easily prepared simply by combining all the ingredients in a suitable vessel or container. The
15 order of mixing the ingredients is not particularly important and generally the various ingredients can be added sequentially or all at once or in the form of aqueous emulsions of each or all of the primary surfactants and co-surfactants can be separately prepared and combined with each other. It is important to note that emulsions of, for instance, organic acid emulsions would not be acceptable for use in the present invention, since
20 such emulsions would be a strong skin irritant and counterproductive to the intended use of the present invention. The protein, when present, can be added as an aqueous emulsion thereof or can be added directly. It is not necessary to use elevated temperatures in the formation step and room temperature is sufficient. However, higher temperatures of up to about 180°F (82.2°C), preferably 110 to 140°F (43.3 to 60°C), can
25 also be used.

For administration to the skin of a human or other mammal, the treatment compositions will often be sterilized or formulated to contain one or more preservatives for

incorporation into pharmaceutical, cosmetic or veterinary formulations. These treatment compositions can be sterilized by conventional, well-known sterilization techniques, e.g., boiling or pasteurization, without substantially adversely affecting the biological activity of the composition. The compositions may contain pharmaceutically acceptable auxiliary
5 substances as required to approximate physiological conditions and as necessary to prepare compositions for convenient administration, such as pH adjusting and buffering agents, preservatives, and delivery vehicles. Actual methods for preparing pharmaceutically administrable compounds will be known or apparent to those skilled in the art and are described in detail in, for example, Remington's Pharmaceutical Science,
10 supra.

Perfumes, dyes and pigments can also be incorporated into the treatment compositions of the invention. For semi-solid compositions, as would be appropriate for pastes and creams intended for topical administration, the peptone-copper complexes can be provided separately or may be compounded with conventional nontoxic carriers such
15 as, for example, aloe vera gel, squalene, glycerol stearate, polyethylene glycol, cetyl alcohol, stearic acid, and propylene glycol, among others. Such compositions may contain about 5-100% active ingredients, more preferably about 5-25%.

The compositions formulated for administration to the skin are administered to a wearer, such as humans, with un-compromised skin or in situations where a subject is
20 already suffering from damaged skin (e.g., peeling) due to ultraviolet or other irradiation or oxidative skin damage. The treatment compositions are administered in an amount sufficient to allow inhibition of further damage by topically administered irritating substances or other unknown irritating substances and are more effective than if the host were not treated. Amounts adequate to accomplish these effects are defined as a
25 "therapeutically effective dose" and will vary according to the application.

In prophylactic and cosmetic applications the compositions are employed for protecting the skin from damage. Thus, the skin health benefit agents and/or silk proteins

are administered to a host under conditions which protect the integrity of the skin, maintains physiological pH, skin hydration and lubrication. In these uses, the precise amounts again depend on the amount of protection desired and the extent and conditions under which the skin is exposed to potentially damaging conditions, such as those caused by fecal and urine proteases, or other irritating substances. They can generally range from about 0.1 mg to about 10 mg per day per square centimeter of skin. Single or multiple administrations of the compositions can be carried out daily or over a prolonged period of time.

The silk proteins of the invention may be administered to the skin in relatively large amounts without serious side effects, although indiscriminate use may produce irritation of the skin. In instances where the compositions are administered prophylactically to inhibit oxidative or biochemical damage to the skin or to those suffering from only mild skin damage, irritation or inflammation of the skin, the dose may be adjusted to lower maintenance levels.

The treatment composition providing skin protection and enhanced repair of the present invention, including pharmaceutical compositions, may be administered alone or as combination or adjunct therapy or prophylaxis. For example, the treatment compositions can be used in combination with other skin protective factors or those found to improve other aspects of protection or healing. In this manner a synergistic effect may be attained that yields a clinical efficacy greater than that realized with any single factor.

Further, while the compositions described herein stimulate a spectrum of skin protective processes, skin can differ considerably in its properties, leading one to utilize a combination of a composition described herein and another compound or factor.

Factors with reported healing properties which can be included with the silk protein compositions for use in protective/healing formulations and methods of the present invention include, for example, epidermal growth factor, fibroblast growth factor, nerve growth factor, transforming growth factors, angiogenic growth factors, heparin, fibronectin,

fibrin, platelet-derived growth factor, enzymatic superoxide dismutase, extracts of blood or factors from the blood, and other similar factors.

Substrates particularly adapted to receive exudate liquids such as menses, mucous, blood products, feces, and others, which will be apparent to those skilled in the art, are useful in the present invention. As used herein, the term "substrate" refers to a material that can be a woven fabric, knit fabric, nonwoven fabric, foam, film-like material (e.g. an apertured film-like material) or paper material. Particularly useful substrates include infant and child care products such as disposable diapers, training pants and baby wipes, feminine hygiene products such as menses absorbing devices like sanitary napkins and tampons, bandages, and incontinent products, for example. In accordance with the invention the substrate is often normally hydrophobic and contains a treatment composition placed so as to contact the exudate. Many polymers useful in the formation of nonwoven webs, e.g. polypropylene, are hydrophobic and highly apolar. As is known to those skilled in the art, sericin is highly hydrophilic, hence it is polar. There is, therefore, no affinity between the substrate and the silk protein, making it difficult to apply the silk protein to the substrate. The unique combination of the silk protein with the surfactants of the present invention overcomes this lack of affinity. In other words, the surfactant serves as a medium to carry the silk protein for application to the substrate. As would be understood by those skilled in the art, some synthetic fibers, such as nylon, are hydrophilic, and would not therefore have this particular problem addressed with the present invention.

Advantageously the substrate is a nonwoven web and may be, for example, a spunbond, meltblown, coformed or bonded carded web. Additional substrates which can be used include foams and films that are fibrillated, apertured or otherwise treated to have fiber-like properties as well as laminates of these and/or nonwoven webs. Depending on the particular application, the substrate may be used as a body contact liner, a distribution

layer between a liner and an absorbent layer, an absorbent layer, or in more than one of these layers.

As used herein the term "nonwoven fabric or web" means a web having a structure of individual fibers or threads, which are interlaid, but not in a regular or identifiable manner as in a knitted fabric. The term also includes individual filaments and strands, yarns or tows as well as foams and films that have been fibrillated, apertured, or otherwise treated to impart fabric-like properties. Nonwoven fabrics or webs have been formed from many processes such as for example, meltblowing processes, spunbonding processes, coforming processes and bonded carded web processes. The basis weight of nonwoven fabrics is usually expressed in ounces of material per square yard (osy) or grams per square meter (gsm) and the fiber diameters useful are usually expressed in microns. (Note that to convert from osy to gsm, multiply osy by 33.91).

As used herein the term "microfibers" means small diameter fibers having an average diameter not greater than about 50 microns, for example, having an average diameter of from about 0.5 microns to about 50 microns, or more particularly, microfibers may have an average diameter of from about 2 microns to about 40 microns. Another frequently used expression of fiber diameter is denier, which is defined as grams per 9000 meters of a fiber and may be calculated as fiber diameter in microns squared, multiplied by the density in grams/cc, multiplied by 0.00707. A lower denier indicates a finer fiber and a higher denier indicates a thicker or heavier fiber. For example, the diameter of a polypropylene fiber given as 15 microns may be converted to denier by squaring, multiplying the result by 0.89 g/cc and multiplying by 0.00707. Thus, a 15 micron polypropylene fiber has a denier of about 1.42 ($15^2 \times 0.89 \times 0.00707 = 1.415$). Outside the United States the unit of measurement is more commonly the "tex", which is defined as the grams per kilometer of fiber. Tex may be calculated as denier/9.

As used herein the term "spunbonded fibers" refers to small diameter fibers which are formed by extruding molten thermoplastic material as filaments from a plurality of fine.

usually circular capillaries of a spinneret with the diameter of the extruded filaments then being rapidly reduced as, for example, described in U.S. Patent No. 4,340,563 to Appel et al., and U.S. Patent No. 3,692,618 to Dorschner et al., U.S. Patent No. 3,802,817 to Matsuki et al., U.S. Patent Nos. 3,338,992 and 3,341,394 to Kinney, U.S. Patent No. 3,502,763 to Hartmann, U.S. Patent No. 3,502,538 to Levy, and U.S. Patent No. 3,542,615 to Dobo et al.

Spunbond fibers are quenched and generally not tacky when they are deposited onto a collecting surface and usually subjected to a separate bonding step. Spunbond fibers are generally continuous and have average diameters frequently larger than 7 microns, more particularly, between about 10 and 20 microns.

As used herein the term "meltblown fibers" means fibers formed by extruding a molten thermoplastic material through a plurality of fine, usually circular, die capillaries as molten threads or filaments into converging high velocity, usually heated, gas (e.g. air) streams which attenuate the filaments of molten thermoplastic material to reduce their diameter, which may be to microfiber diameter. Thereafter, the meltblown fibers are carried by the high velocity gas stream and are deposited on a collecting surface often while still tacky to form a web of randomly dispersed meltblown fibers. Such a process is disclosed, for example, in U.S. Patent No. 3,849,241 to Butin. Meltblown fibers are microfibers which are usually continuous, but which may also be discontinuous, and are generally smaller than 10 microns in average diameter.

The substrate of the present invention may also include a bonded carded web. As used herein "bonded carded webs" or "BCW" refers to nonwoven webs formed by carding processes as are known to those skilled in the art and further described, for example, in coassigned U.S. Patent No. 4,488,928 to Alikhan and Schmidt which is incorporated herein by reference in its entirety. Briefly, carding processes involve starting with a blend of, for example, staple fibers with bonding fibers or other bonding components in a bulky batt that is combed or otherwise treated to provide a generally uniform basis weight. This web is

heated or otherwise treated to activate the adhesive component resulting in an integrated, usually lofty nonwoven material.

As would be understood by one of ordinary skill in the art, such nonwoven webs may be formed from different types of polymers, which may be extruded as monocomponent fibers, biconstituent fibers and/or conjugate fibers (multi- and bicomponent fibers) filaments. Biconstituent fibers are sometimes also referred to as multiconstituent fibers. Fibers of this general type are discussed in, for example, U.S. Patent No. 5,108,827 to Gessner. Bicomponent and biconstituent fibers are also discussed in the textbook Polymer Blends and Composites by John A. Manson and Leslie H. Sperling, copyright 1976 by Plenum Press, a division of Plenum Publishing Corporation of New York, ISBN 0-306-30831-2, at pages 273 through 277. Conjugate fibers are taught in U.S. Patent No. 5,108,820 to Kaneko et al., U.S. Patent No. 5,336,552 to Strack et al., and U.S. Patent No. 5,382,400 to Pike et al.

Small amounts of additives may be added for color, anti-static properties, lubrication, hydrophilicity, antibacterial, antimold, deodorizing effect, and the like. These additives, e.g. titanium dioxide for color and chitosan as an antibacterial, are generally present in an amount less than 5 weight percent and more typically about 2 weight percent.

As used herein, the term "personal care product" includes diapers, training pants, swim pants, absorbent underpants, adult incontinence products, sanitary wipes, feminine hygiene products such as sanitary napkins and tampons, wound dressings and bandages.

As used herein, the term "hydrophilic" means that the polymeric material has a surface free energy such that the polymeric material is wettable by an aqueous medium, i.e. a liquid medium of which water is a major component. The term "hydrophobic" includes those materials that are not hydrophilic as defined. Hydrophobic materials may be treated internally or externally with surfactants and the like to render them hydrophilic.

The substrate of the present invention may be a multilayer laminate. An example of a multilayer laminate is an embodiment wherein some of the layers are spunbond and

some meltblown such as a spunbond/meltblown/spunbond (SMS) laminate as disclosed in U.S. Patent No. 4,041,203 to Brock et al., U.S. Patent No. 5,169,706 to Collier, et al, and U.S. Patent No. 4,374,888 to Bornslaeger. Such substrates usually have a basis weight of from about 0.1 to 12 osy (6 to 400 gsm), or more particularly from about 0.75 to about 3
5 osy.

Spunbond nonwoven fabrics are generally bonded in some manner as they are produced in order to give them sufficient structural integrity to withstand the rigors of further processing into a finished product. Bonding can be accomplished in a number of ways such as hydroentanglement, needling, ultrasonic bonding, adhesive bonding, stitchbonding,
10 through-air bonding and thermal bonding such as calendering.

The addition of the treatment composition to the substrate may be accomplished by conventional means such as spraying, coating, dipping and the like although the use of high solids spray is advantageous in cases where drying and/or compression is desired to be minimized. The amount of the treatment composition used will depend on the
15 particular end use as well as factors such as basis weight and porosity of the substrate. Referring to FIG. 1, an exemplary process will be described for application to one or both sides of a traveling substrate. It will be appreciated by those skilled in the art that the invention is equally applicable to inline treatment or a separate, offline treatment step. Substrate 12, for example a spunbond or meltblown nonwoven web is directed over support
20 rolls 15,17 to a treating station including rotary spray heads 22 for application to one side 14 of web 12. An optional treating station (shown in phantom) which may include rotary spray heads 18 can also be used to apply to opposite side 23 of substrate 12. Each treatment station receives a supply of treatment composition 30 from a reservoir (not shown). The treated substrate may then be dried if needed by passing over dryer cans 25 or other drying
25 means and then wound as a roll or converted to the use for which it is intended. Alternative drying means include ovens, through air dryers, infra red dryers, air blowers, and the like.

As referred to above, a unique and surprising aspect of the present invention includes the ability of the treatment composition to be transferred from the substrate to the skin. It has been found that when a liquid is introduced to the substrate, the treatment composition will dissolve in the liquid, and then liquid-mediated transfer of the treatment composition to the skin occurs. In other words, the treatment composition including the skin health benefit agent dissolves off of the substrate into the liquid, which then deposits the thin, tenacious and substantially continuous film of the skin health benefit agent onto the skin. Urine is an example of a liquid that can transfer the treatment composition from the substrate to the skin. As another example, the liquid generated by the body after abrasion or injury to the skin, might provide sufficient liquid-mediated transfer of the treatment composition from the substrate, in this case, a bandage or wound dressing. In general, when wetness increases, the treatment composition will transfer to the skin to form a protective barrier.

An exemplary article 80, in this case a diaper, is shown in FIG. 2. Referring to FIG. 2, most such personal care absorbent articles 80 include a liquid permeable top sheet or liner 82, a barrier back sheet or outercover 84 and an absorbent core 86 disposed between and contained by the top sheet 82 and back sheet 84. Articles 80, such as diapers, may also include some type of fastening means 88 such as adhesive fastening tapes or mechanical hook and loop type fasteners to maintain the garment in place on the wearer. The substrate 12 may be used to form various portions of the article including, but not limited to the top sheet or liner 82.

The following examples are offered by way of illustration, not by way of limitation.

EXAMPLES

The compositions of the present invention were formed by creating an emulsion of a skin health benefit agent and water as the carrier liquid. Aqueous emulsions of zinc salt as the skin health benefit agent, AHCVEL, as the surfactant system, and, in some instances, SERICIN and CROSILK as the silk protein, were prepared. The stable

emulsions were diluted to about a 5% by weight emulsion and applied to the surface of a polyolefin nonwoven liner fabric at 3 and 6% by weight via a saturation dip and squeeze process as described in more detail below. The fabrics were then tested for fluid intake rate, softness, skin transfer, skin barrier, anti-inflammatory, and the like, as describe in
5 more detail below.

Fluid Intake Rate: This test is identified as Fluid Strikethrough EDANA 150.1-90 and measures the time taken for a known volume of liquid (simulated urine) applied to the surface of a nonwoven test sample in contact with an underlying absorbent pad to pass through the nonwoven. In general, a 50 ml burette is positioned on a ring stand with the
10 tip inside a funnel. A standard absorbent pad of 5 plies of specified filter paper (482% absorbency) is placed on an acrylic glass base plate below the funnel, and a nonwoven sample is placed on top of the absorbent. An acrylic glass strike-through plate 25 mm thick and weighing 500 g is placed over the sample with the cavity centered 5 mm below the funnel. The burette is filled with liquid, keeping the funnel closed, and a quantity of
15 the liquid (e.g., 5 ml or 10 ml) is run into the funnel. The 5 ml or 10 ml is allowed to discharge starting a timer which stops when the liquid has penetrated into the pad and fallen below a set of electrodes, and the elapsed time is recorded. The liquid used was Blood Bank Saline, available from Stephens Scientific Co., Catalog No. 8504.

Softness Test: Although a specific softness test was not conducted, each of the
20 samples prepared were noted to have a "soft hand", and would be suitable for use in articles as described herein.

Skin Transfer Test: Sericin was labeled with the fluorescent dye, Texas Red (TR), according to the procedure suggested by Molecular Probes, Inc., Eugene OR (Texas Red protein labeling kit F-6162). A 4.5 wt.% emulsion of sericin, 200uL, was
25 reacted with 100uL of a 5mg/mL solution of Texas Red succinimide in dimethylsulfoxide for 1 hour. The thus labeled protein was isolated via gel chromatography with centrifugation for 3 minutes at 1100 x g.

Emulsions of Ahcovel containing a mixture of TR labeled and unlabelled sericin was used to treat a substrate of 0.6 osy polypropylene spunbond nonwoven web, prepared as a diaper liner according to the procedure described. Fluorescent microscopy using a high pressure Hg lamp with excitation and emission bandpass filters of 595nm and 615nm, respectively, confirmed the presence of the labeled protein on the substrate. FIG. 3 is a 200X optical microscopy (Brightfield and Fluorescent Image) of the substrate treated with the treatment composition according to the present invention. The untreated substrate showed no significant fluorescence, (not shown).

Upon exposing the treated substrate to water, intense fluorescence, characteristic of TR, could be detected in solution, suggesting solubility of the labeled protein into the wetting media. Sericin was removed from the substrate due to its solubility in water. FIG. 4 is a 200X optical microscopy (Fluorescent Image) showing this liquid mediated transfer of the treatment composition from the treated substrate and dissolving in the liquid.

Transfer of the labeled protein from the treated substrate to the skin was verified by the following procedure. A 1 cm² square of treated substrate was secured to the volar forearm with adhesive tape. A majority of the area of the substrate was not covered with tape, which was placed only on the perimeter to secure the substrate to the arm. The substrate was subsequently wetted with 300uL of water. After several minutes, the substrate was removed from the arm, and the stratum corneum previously covered by the material was tape stripped with Dsquame tape to remove superficial layers of skin for fluorescent microscopic analysis. Microscopy at 100x and 200x clearly revealed intense fluorescence associated with the TR labeled protein, thus confirming that transfer from the substrate to the skin, mediated by wetting, was conclusive. FIG. 5 shows the thin, tenacious, substantially continuous film of silk protein that was applied to the skin by means of the present invention. The morphology of the resulting protein coating was consistent with a thin, tenacious, substantially continuous film of the sericin protein on the

corneocytes of the forearm. FIG. 6, on the other hand, shows the variability of application of the silk protein by means of mechanical transfer described in the prior art.

The fabrics were then tested for topical delivery of the zinc salt using excised skins in modified FRANZ diffusion cells. The amount of zinc accumulating and penetrating the skin was determined over a 36 hour period. The accumulation of zinc was determined to be 1311 $\mu\text{g/g}$ dry weight of skin, while the penetration was measured to be 3.2 $\mu\text{g}/\text{cm}^2/\text{hr}$.

Skin Barrier Test: Uniformity of the barrier film on the skin as shown in FIG. 5 was indicative of good barrier properties.

Anti-inflammatory: The effect of skin health benefit agent to inhibit the hydrolysis of a model protein substrate by urine and a fecal extract was determined. In addition, the ability of the skin health benefit agent to reduce a fecal extract-elicited pro-inflammatory response in EpiDerm™ was measured. A silk protein applied to a material was also placed on the EpiDerm™ sample, both before and after application of the fecal irritant and evaluated for its ability to reduce a fecal-extract pro-inflammatory response. The release of a pro-inflammatory signaling molecule Interleukin-1 alpha, was compared to that of the control not containing the skin health benefit agent.

Inhibition of the Proteolytic Activity of Fecal Extract

Aqueous zinc salts were shown to inhibit a chemical reaction that contributes to diaper rash. The proteolytic activity of a fecal extract was measured using fluorescently labeled casein. Inhibiting emulsions of zinc sulfate heptahydrate in water were prepared ranging from 0-1 mM.

A fecal extract sample was prepared from feces obtained from an infant on antibiotics (Sulfatrim) who had diaper rash. To prepare the extract, the feces was suspended in water and vigorously vortexed. After vortexing, the sample was held on ice prior to centrifugation at 15,000 times the force of gravity for 20 minutes. The supernatant was filtered through 0.22 micron cellulose acetate filters and stored at -80°C until use.

Trypsin (molecular weight = 23,500 daltons), a protease known to contribute to diaper rash, was measured in the fecal extract at a concentration of 5,850 picomoles/milliliter. Pancreatic elastase (molecular weight = 25,000 daltons), a suspected contributor, was measured in the fecal extract at a concentration of 83.6 picomoles/milliliter. The fecal
5 extract (7.1 mg/ml in water) was diluted in water to 2 µg/ml.

The zinc sulfate heptahydrate (Aldrich Chemicals, WI) solutions (20 µL) having a molecular weight of 287.5 were added to wells of a 96 white plate (Dynex, Chantilly, VA) containing 100 µL of a fecal extract and allowed to incubate for 15 minutes at room temperature. The reaction was initiated with the addition of 80 µL of 12.5 µg/ml solution
10 of a fluorescent dye-labeled casein substrate (EnzChek Protease Assay Kit (E-6639), Molecular Probes, Eugene, OR) in 20 mM Tris-HCl, pH 8.0. Reaction of the fecal extract with the casein substrate cleaves the fluorescent dye from the substrate. Relative fluorescence units (RFUs) were collected using the Fluoroskan Ascent System (Labsystems, Incorporated, Needham Heights, MA) with excitation and emission filters of
15 485 and 538 nm, respectively. Data were collected each minute for 15 minutes and rates (RFU/min) were calculated. Using the uninhibited wells as 100% protease activity, percent of fecal proteolytic activity remaining was determined for each concentration of zinc inhibitor ($\text{Inhibited Rate}/\text{Uninhibited rate} \times 100$).

The data show that zinc effectively inhibited the hydrolysis of casein by the fecal
20 extract in a dose-dependent manner. A plot of the data for the fecal extract sample is shown in FIG. 7, which is a graph showing how the proteolytic activity of fecal extract was reduced as the zinc sulfate heptahydrate concentration was increased. These data show that the aqueous zinc emulsion has the ability to neutralize proteases in feces that have been implicated to induce skin inflammation in the diaper environment (Anderson, P.H.,
25 Bucher, A.P., Saees, I., Lee, P.C., Davis, J.A., and Maibach, H. I. Faecal enzymes: *in vivo* skin irritation. *Contact Dermatitis* 1994; 30, 152-158).

Inhibition of the Proteolytic Activity of Infant Urine

Aqueous zinc salts were shown to inhibit a chemical reaction that contributes to diaper rash. The proteolytic activity of infant urine was measured using fluorescently labeled casein. Inhibiting solutions of zinc sulfate heptahydrate in water were prepared
5 ranging from 0-50 mM.

Two (2) infant urine (100 μ L) samples were added to wells of a 96 white plate (Dynex) containing 80 μ L of 12.5 μ g/ml solution of a fluorescent dye-labeled casein substrate (EnzChek Protease Assay Kit (E-6639)) in 100 mM Tris-HCl, pH 8.0, and allowed to incubate for 60 minutes at 37°C. The reaction was initiated with the addition of
10 20 μ L of zinc sulfate heptahydrate in water, ranging from 0-50 mM. Reaction of the infant urine with the casein substrate cleaved the fluorescent dye from the substrate. RFUs were collected using the Fluoroskan Ascent System with excitation and emission filters of 485 and 538 nm, respectively. Data were collected each minute for 60 minutes at 37°C and rates (RFU/min) from 30-50 minutes were calculated for each zinc concentration.
15 Using the uninhibited wells as 100% protease activity, percent of urine proteolytic activity remaining was determined for each concentration of zinc inhibitor (Inhibited Rate/Uninhibited rate* 100).

The data show that zinc effectively inhibited the hydrolysis of casein by infant urine in a dose-dependent manner. A plot of the data is shown in FIG. 8, which is a graph
20 showing how the proteolytic activity of infant urine was reduced as the zinc sulfate heptahydrate concentration was increased. These data show that the zinc aqueous zinc solution had the ability to neutralize proteases in infant urine.

Inhibition of the Proteolytic Activity of Fecal Extract on Synthetic Skin

Zinc sulfate heptahydrate was shown to inhibit the reaction of fecal extract with
25 synthetic skin. The synthetic skin, EpiDerm™ 201 (MatTek Corporation, Ashland, MA) contains keratinocytes that release interleukin-1 alpha (IL-1 alpha) when subjected to

proteases in the fecal extract. When the IL-1 alpha was released, it diffused from the skin into the fluid below the EpiDerm™. Samples of this fluid were taken and analyzed for the presence of the IL-1 alpha. Higher levels of IL-1 alpha was indicative of greater skin irritation.

5 Prior to application onto the EpiDerm™, the fecal extract (10.4 μ L) was pre-incubated for 30 minutes at room temperature with 250 mM zinc sulfate heptahydrate in water (2.6 μ L). Water only and fecal extract only samples served as controls. After application of the samples to the EpiDerm™, 25 μ L aliquots were removed from the underlying media at 8, 12, and 24 hours to test for the presence of the IL-1 alpha. The
10 aliquots were directly added to a 1.5 mL micro-centrifuge tube containing 225 μ L of filtered-sterilized 20 mM tris-HCl, pH 8.0, 1% BSA buffer and stored at -80°C . After all samples were collected, IL-1 alpha levels were quantified using the R&D Systems Interleukin-1 alpha Quantikine Kit (R&D Systems, Minneapolis, MN).

 A plot of the data is shown in FIG. 9, which is a graph showing that the addition of
15 the zinc sulfate heptahydrate (FE + zinc) reduced the amount of interleukin-1 alpha released into the underlying media relative to the application of the substrate treated with uninhibited fecal extract (FE + water). A similar reduction was shown at 8, 12, and 24 hours. The asterisk over the error bars in FIG. 9 represent a Student t-test 95% confidence interval.

20 Re-running the same experiment with varied concentrations of zinc sulfate heptahydrate (0, 25, 50, 125, and 250 mM) in the 2.6 μ L aliquot added to the 10.4 μ L sample of fecal extract, demonstrated that zinc sulfate heptahydrate effectively inhibits the proteolytic activity of fecal extract in a dose-dependent manner. A plot of the data, shown in FIG. 10, shows that the proteolytic activity of fecal extract is reduced as the zinc sulfate
25 heptahydrate concentration is increased.

Effect of Zinc Salt on pH of Urine and Fecal Extract

- Increased concentration of zinc sulfate heptahydrate reduced the pH of infant urine and fecal extract as shown below in Table 1. The pH of the samples was measured using pH Test Strips 4.5-10.0 (P-4536, Lot 067H1346, Sigma Chemical Co., St. Louis, MO). The pH of the infant urine samples were reduced by 1.5-2.0 pH units and the pH of the fecal extract sample was reduced by 1 pH unit.

Table 1 – Effect of Zinc Concentration on pH

Zinc Sulfate Heptahydrate Concentration mM	Infant Urine Example F pH	Infant Urine Example G pH	Infant Urine Example H pH	Fecal Extract Example B pH
0.0	8.5	7.0	8.0	6.5
.1	8.5	7.0	8.0	6.5
1.0	8.0	7.0	7.5	6.5
2.5	7.5-8.0	6.5	7.0-7.5	6.0-6.5
5.0	7.5	5.5	7.0	6.0
10.0	7.0	5.0	6.5	5.5
20.0	7.0	5.0	6.5	5.5

Treatment compositions

- 10 Treatment compositions containing water, AHCOVEL, SERICIN, CROSILK, and zinc sulfate monohydrate were prepared according to Table 2 below. For comparison purposes, a control composition was prepared containing only water and AHCOVEL.

Table 2 – Composition Concentrations (wt.%)

Compositions	Water	AHCOVEL	SERICIN	CROSILK 10,000	Zinc Sulfate Monohydrate
Control	80	20	-	-	-
1	69.1	20	-	10	.9
2	74.1	20	5	-	.9
3	69.1	20	10	-	.9
4	59.1	20	20	-	.9

Although not specifically shown in the data, silk protein emulsions, without the aid of surfactant, were applied to a substrate. Since the protein is hydrophilic, and the substrate is hydrophobic, the protein would not easily wet and saturate the substrate, and instead formed a bead on top of the substrate.

5 Permeation of Zinc from Lotion to Skin

The permeation of zinc from aqueous lotions containing 1% zinc sulfate monohydrate into and through human cadaver skin and EpiDerm™ (EPI-612, MatTek Corporation, Ashland, MA) was assessed. Modified Franz diffusion cells (PermeGear Inc., Riegelsville, PA) were used for the *in vitro* (diffusional areas of 0.64 cm², receptor
10 compartment volume 5.1 ml). The receptor compartment was filled with isotonic saline (0.9% NaCl) containing 0.1% formaldehyde preservative, maintained at 37°C ± 0.5°C, and continuously stirred at 600 rpm. No surfactants were added.

Skins (dermatomed human cadaver skins, female abdominal, and EpiDerm™) were mounted between the donor and receptor compartments in the Franz diffusion cells
15 and clamped. Skins were first allowed to equilibrate for 1 hr. before experimentation. Then, any air bubbles remaining in the receptor cells were removed. At that point, 500 mg of the aqueous emulsion was placed on each skin. All donor cells were occluded with Parafilm®. Receptor samples (1.5 ml) were taken after 36 hours and frozen at -70°C prior to Ion Coupled Spectroscopy (ICP) analysis to determine the zinc flux through the skins.

20 After 36 hours, the skins were removed from the cells and washed briefly in water. All skin samples were dried for 24 hr. at 105°C. The dried skin samples were weighed and dissolved in nitric acid (68%) (EM, NJ, USA), for 96 hours. The mixture was then filtered through a 0.22 micron filter (Millex™- GV, Millipore, Mass, USA). Emulsions were diluted 10-fold or 20-fold before being subjected to ICP analysis for skin zinc content
25 quantification.

The zinc flux and skin content was determined after 36 hr. and are shown in Table 3. The results are expressed as means ± standard deviation. The number of analyzed

samples is 3-4 per experiment. All of the examples retained significant quantities of zinc. EpiDerm™ was more permeable and retained more zinc compared to human cadaver skin.

Table 3: Percutaneous permeation parameters for zinc sulfate monohydrate

Composition (from Table 2)	Human Cadaver Skin		EpiDerm™	
	Skin Content ($\mu\text{g/g}$)	Zinc Flux ($\mu\text{g}/\text{cm}^2\text{hr}$)	Skin Content ($\mu\text{g/g}$)	Zinc Flux ($\mu\text{g}/\text{cm}^2\text{hr}$)
2	2074 \pm 474	6.3 \pm 3.2	26965 \pm 2506	58.2 \pm 15.1
3	2432 \pm 481	4.0 \pm 2.1	39841 \pm 8568	63.5 \pm 27.7
4	2387 \pm 449	8.8 \pm 3.4	29431 \pm 9279	40.1 \pm 7.5

5

Treated Substrate

Untreated polypropylene spunbond materials (basis weight of about 0.5 ounces per square yard) were used as a substrate for the treatment compositions. The compositions were applied to the substrates by a low-solids batch treatment process. An 8 in. x 12 in. (20.32 x 30.48 cm) example of the substrate was first dipped in an aqueous treatment bath of known composition illustrated in Table 4 below.

Table 4 – Treatment Bath Concentration

Example	Composition (from Table 1)	Water	Ahcovel	Sericin	Crosilk 10,000	Zinc Sulfate Monohydrate
1	Control	98.8	1.2	-	-	-
2	Control	97	3	-	-	-
3	Control	94	6	-	-	-
4	1	98.146	1.2	-	.6	.054
5	1	97	1.942	-	.971	.087
6	1	94	3.883		1.942	.175
7	3	97	1.942	.971		.087
8	3	94	3.883	1.942	-	.175

The saturated examples were then nipped between two rubber rollers in a laboratory wringer, Type LW-1, No. LW-83A (Atlas Electric Devices, Chicago, IL), and subsequently dried in an oven at 60°C for about 20 minutes or until constant weight was obtained. Nip pressure was adjusted to achieve a 100% wet pick-up (%WPU). %WPU is calculated from the following equation:

$$\%WPU = [(W_w - W_d)/W_d] \times 100,$$

20

where:

Ww = wet weight of the nipped fabric,

Wd = dry weight of the treated fabric.

Knowing the bath concentration and the %WPU, the % Add On can be calculated

5 from the following equation:

$$\%AddOn = (\%BathConcentration) \times (\%WPU) + 100$$

If, as in this example, the %WPU = 100, then the % Add-On will equal the % Bath Concentration. However, other %WPU and % Bath Concentration combinations can be used to achieve similar results. Final % Add-On for each component of each sample is

10 shown in Table 5 below.

Table 5 – % Add-On for Each Example of Table 4

Example	Ahcovel	Sericin	Crosilk 10,000	Zinc Sulfate Monohydrate
1	1.2	-	-	-
2	3	-	-	-
3	6	-	-	-
4	1.2	-	.6	.054
5	1.942	-	.971	.087
6	3.883	-	1.942	.175
7	1.942	.971	-	.087
8	3.883	1.942	-	.175

The Fluid Intake Rate was determined for each of the examples of Table 5 as shown in Table 6 below, according to the test as described above. As shown in the data,

15 fluid-intake rates have not been adversely affected by addition of the treatment composition of the present invention, and the substrates, therefore, provide adequate fluid handling properties.

Table 6 - Fluid Intake Time (sec.)

Example #	Intake Time (second)				
	Insult 1	Insult 2	Insult 3	Insult 4	Insult 5
2	2.82	2.83	3.05	3.21	3.15
3	2.45	2.43	2.54	2.47	2.65
5	2.38	2.41	2.43	2.35	2.55
6	2.63	2.69	2.60	2.72	2.70
7	2.51	2.72	2.43	2.32	2.38
8	2.44	2.56	2.37	2.38	2.42

Permeation of Zinc from Treated Spunbond to Skin

The permeation of zinc from treated nonwoven spunbond examples 7 and 8 from Table 5 into and through human cadaver skin was assessed. Modified Franz diffusion cells (PermeGear Inc., Riegelsville, PA) were used for the *in vitro* (diffusional areas of 0.64 cm², receptor compartment volume 5.1 ml). The receptor compartment was filled with isotonic saline (0.9% NaCl) containing 0.1% formaldehyde preservative, maintained at 37°C ± 0.5°C, and continuously stirred at 600 rpm. No surfactants were added.

Skins (dermatomed human cadaver skins, female abdominal) were mounted between the donor and receptor compartments in the Franz diffusion cells and clamped. Skins were first allowed to equilibrate for 1 hr before experimentation. Then any air bubbles remaining in the receptor cells were removed. At that point, a circle of the non-woven fabrics was cut out and clamped on top of the skin in each cell. Distilled water (500 µl) was placed on top of each material and the donor cell was occluded with Parafilm®. Receptor samples (1.5 ml) were taken after 36 hours and frozen at -70 °C prior to Ion Coupled Spectroscopy (ICP) analysis to determine the zinc flux through the skins.

After 36 hours, the skins were removed from the cells and washed briefly in water. All skin samples were dried for 24 hr at 105°C. The dried skin samples were weighed and dissolved in nitric acid (68%) (EM, NJ, USA). for 96 hours. The mixture was then filtered through a 0.22 micron filter (Millex™- GV, Millipore, Mass, USA). Emulsions were

diluted 10-fold or 20-fold before being subjected to ICP analysis for skin zinc content quantification.

The zinc flux and skin content was determined after 36 hr and are shown in Table 7. The results are expressed as means \pm standard deviation. The number of analyzed examples is 3-4 per experiment. All of the examples retained significant quantities of zinc.

Table 7: Percutaneous permeation parameters for zinc sulfate monohydrate

Example (from Table 5)	Human Cadaver Skin	
	Skin Content ($\mu\text{g/g}$)	Zinc Flux ($\mu\text{g}/\text{cm}^2\text{hr}$)
7	1311 \pm 378	3.2 \pm 1.1
8	1358 \pm 394	3.8 \pm 1.6

10

Inhibition of the Proteolytic Activity of Fecal Extract

The treated spunbond materials were tested to assess their capacity to inhibit the reaction of fecal extract with synthetic skin. The fecal extract was prepared from feces obtained from an infant on antibiotics (Sulfatrim) who had diaper rash. To prepare the extract, the feces were suspended in water and vigorously vortexed. After vortexing, the samples were held on ice prior to centrifugation at 15,000 times the force of gravity for 20 minutes. The supernatant was filtered through 0.22 micron cellulose acetate filters and stored at -80°C until use. Trypsin (molecular weight = 23,500 daltons), a protease known to contribute to diaper rash, was measured in the fecal extract at a concentration of 5,850 picomoles/milliliter. Pancreatic elastase (molecular weight = 25,000 daltons), a suspected contributor, was measured in the fecal extract at a concentration of 83.6 picomoles/milliliter.

20

The synthetic skin, EpiDerm™ 201 (MatTek Corporation, Ashland, MA) contains keratinocytes that release interleukin-1 alpha (IL-1 alpha) when subjected to proteases such as trypsin and pancreatic elastase. When the IL-1 alpha is released, it diffuses from

the skin into the fluid below the EpiDerm™. Samples of this fluid are taken and analyzed for the presence of the IL-1 alpha. Higher levels of IL-1 alpha are indicative of greater skin irritation.

To perform the experiment, 10 µL of water was applied to the surface of the synthetic skin. The treated spunbond materials were cut into about 0.9 cm discs which were placed on top of the water on the EpiDerm™. After about a two hour incubation at about 37 °C, the discs were removed and the EpiDerm™ was insulted with 15 µL of insult fluid where each of the treated spunbond discs had been. A second treated spunbond disc was then placed on top of the insult fluid to simulate the diaper environment. After 11 hours at 37°C, an aliquot from the underlying fluid bathing the EpiDerm™ was removed and the amount of IL-1 alpha quantified using the R&D Systems Interleukin-1 alpha Quantikine Kit (R&D Systems, Minneapolis, MN).

Four treatments were done using the two examples from Table 5 and two insult fluids (fecal extract and water) as shown in Table 8.

Table 8: EpiDerm™ treatments

Code	Example Used	Insult Fluid
I	1 (Control)	Water
J	1 (Control)	Fecal Extract
K	4	Water
L	4	Fecal Extract

The results of the experiments of Table 8 are shown below in Table 9. The application of the materials treated with Crosilk and Zinc (Code K) to the EpiDerm™ reduced the amount of interleukin-1 alpha released into the underlying media relative to the application of the materials treated with just Ahcovel (Code I).

Table 9: IL-1 alpha Released (picograms/milliliter)

Code	Mean	Standard Deviation	Number of samples
I	14.03	4.768	6
J	105	66	5
K	6.77	6.77	6
L	44.92	23.4	6

While various patents and other reference materials have been incorporated herein by reference, to the extent there is any inconsistency between incorporated

5 material and that of this written specification, the written specification shall control. In addition, while the present invention has been described in connection with certain preferred embodiments, it is to be understood that the subject matter encompassed by way of the present invention is not to be limited to those specific embodiments. On the contrary, it is intended for the subject matter of the invention to include all alternatives,

10 modifications and equivalents as can be included within the spirit and scope of the following claims.

We Claim:

1. A treatment combination for imparting a liquid-mediated transfer medium to a substrate, said combination comprising a surfactant and a skin health benefit agent;
the surfactant including a compound selected from the group comprising ethoxylated hydrogenated fatty oils, monosaccharides, monosaccharide derivatives, polysaccharides, polysaccharide derivatives and combinations thereof.
- 5
2. The treatment combination of Claim 1, wherein said skin health benefit agent comprises aqueous zinc salt or zinc sulfate monohydrate.
3. The treatment combination of Claim 2, said surfactant and skin health benefit agent are combined in an aqueous emulsion to form a treatment composition
4. The treatment combination of Claim 2, wherein said surfactant and zinc salt form a treatment composition and are present at a weight ratio of about 0.01-25 wt.% zinc salt to about 75-99.99 wt.% surfactant.
- 5: The treatment combination of Claim 4, wherein said emulsion further comprises 0.1 to 40 wt.% total solids.
6. The treatment composition of Claim 5 further comprising a protein.
7. The treatment composition of Claim 6 wherein said protein further comprises a silk protein.
8. A substrate treated with a treatment combination, said treatment combination

comprising a surfactant and a skin health benefit agent;

the surfactant including a compound selected from the group consisting of
ethoxylated hydrogenated fatty oils, monosaccharides, monosaccharides derivatives,

5 polysaccharides, polysaccharide derivatives, and combinations thereof,

wherein said surfactant is a liquid-mediated transfer medium for transfer of said
skin health benefit agent from said substrate to a wearer.

9. The treated substrate of claim 8 wherein said skin health benefit agent further
comprises zinc salt or zinc sulfate heptahydrate.

10. The treated substrate of Claim 9, wherein the substrate is selected from
woven fabrics, knit fabrics, nonwoven fabrics, foams, film-like materials and paper
materials.

11. The treated substrate of Claim 10, wherein the substrate comprises a
nonwoven web.

12. The treated substrate of Claim 11 wherein said nonwoven web is hydrophilic.

13. The treated substrate of Claim 12, wherein said nonwoven web comprises a
spunbond web, meltblown web, coformed web or bonded carded web.

14. The treated substrate of Claim 13, wherein said substrate comprises a
multilayer laminate.

15. The treated substrate of Claim 11, wherein the treatment combination further
comprises a protein.

16. The treatment combination of Claim 15 wherein said protein further comprises a silk protein.

17. The treated substrate of Claim 16, wherein the combination is applied at a level of about 0.1-1.5% by weight surfactant relative to the basis weight of the substrate.

18. The treated substrate of Claim 17, wherein the combination is applied at a level of about 0.1-1.0% by weight surfactant relative to the basis weight of the substrate.

19. The treated substrate of Claim 18, wherein the combination is applied at a level of about 0.1-0.5% by weight surfactant relative to the basis weight of the substrate.

20. A personal care product comprising the treated substrate of Claim 11.

21. The personal care product of Claim 20, wherein the personal care product is selected from a diaper, training pant, absorbent underpant, adult incontinence product, sanitary wipe, feminine hygiene product, wound dressing and bandage.

22. The personal care product of Claim 21 wherein said skin health benefit agent lowers pH of the personal care product environment

23. The treated substrate of Claim 11, wherein a fluid intake rate of said substrate is not adversely affected by addition of said treatment combination.

24. The method of transferring a thin, tenacious, substantially continuous film of skin health benefit agent to skin comprising:

- a) providing a substrate;
- b) applying a treatment composition to said substrate, said composition
5 comprising a surfactant and a skin health benefit agent in combination; the
surfactant including a compound selected from the group consisting of
ethoxylated hydrogenated fatty oils, monosaccharides, monosaccharide
derivatives, polysaccharides, polysaccharide derivatives, and combinations
thereof,
- 10 c) insulating said substrate with an aqueous based liquid whereby said treatment
composition dissolves in said liquid, and
- d) transferring said treatment composition from said substrate to the skin, thereby
forming a thin, tenacious, substantially continuous, film on the skin.

25. The method of Claim 24 wherein said substrate is a hydrophilic nonwoven
web.

26. The method of Claim 25, wherein the substrate comprises a personal care
product.

27. The method of Claim 25 wherein said skin health benefit agent further
comprises aqueous zinc salt or aqueous zinc sulfate heptahydrate.

28. The method of Claim 27 further comprising addition of a protein to said
substrate.

29. The method of Claim 28 wherein said protein further comprises a silk protein.

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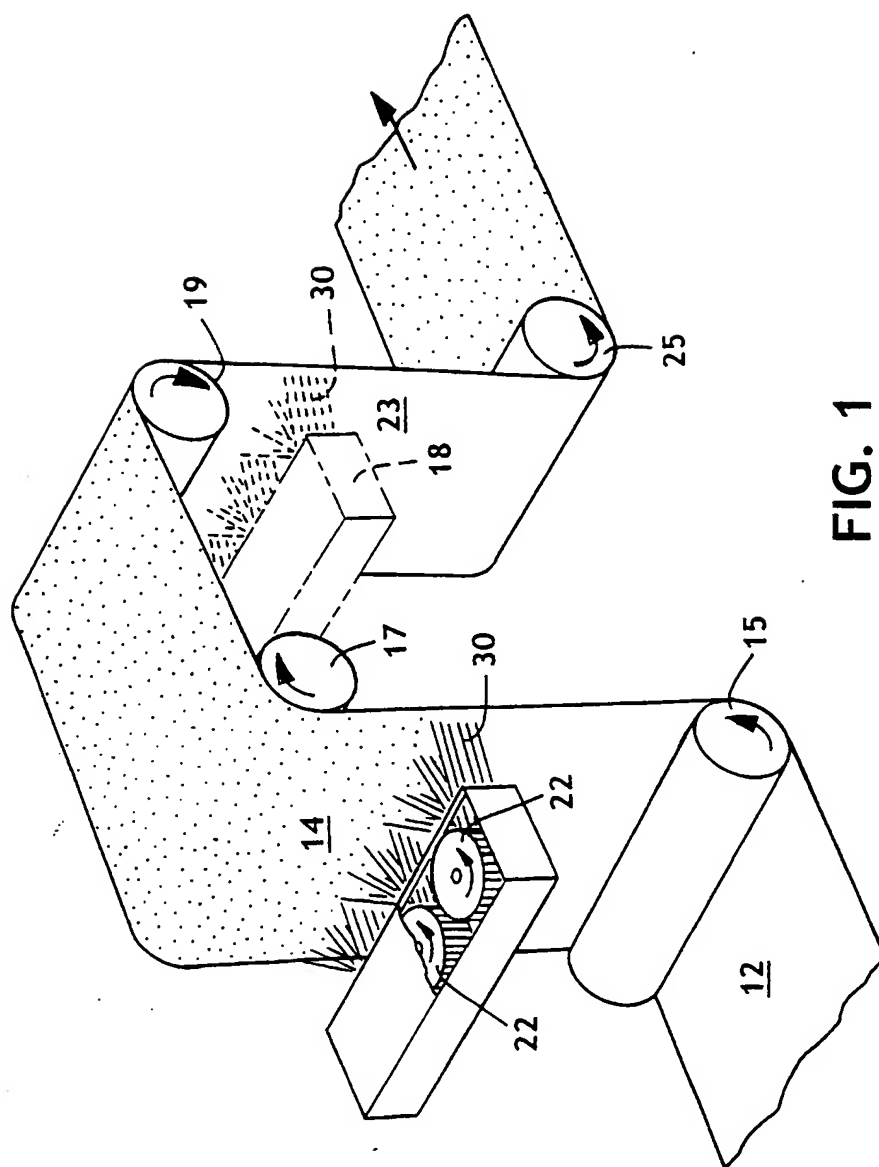


FIG. 1

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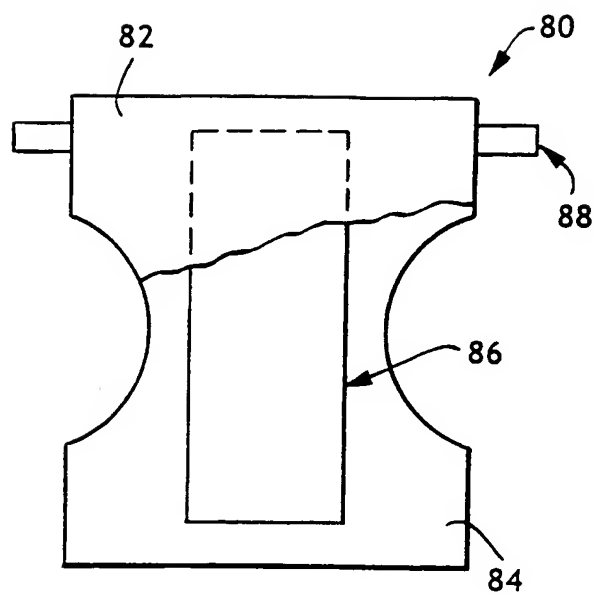


FIG. 2

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Sericin Treated Nonwoven
(a) Brightfield and (b) Fluorescent Image, both at 200x



(a)

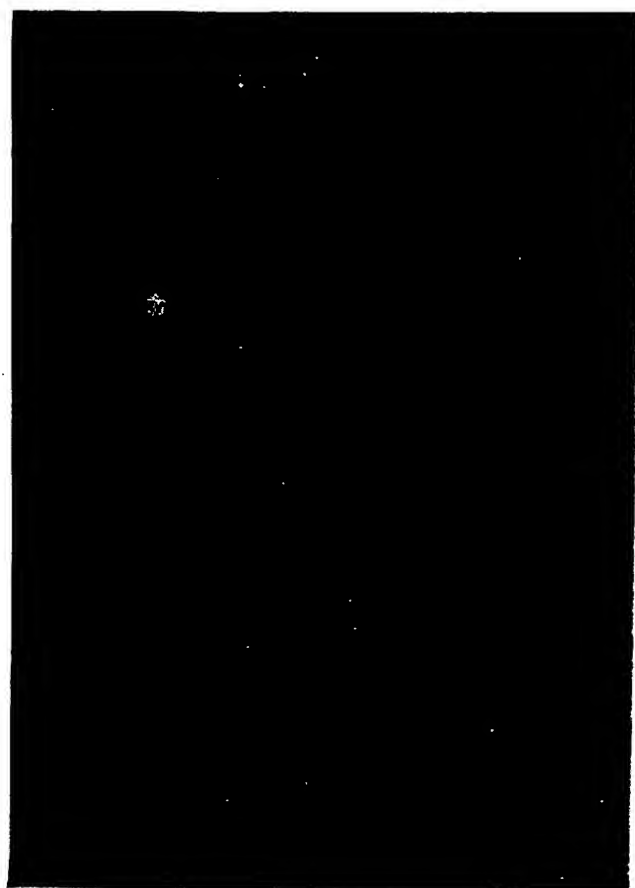


(b)

FIG. 3

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Fluid Mediated Transfer of Sericin
Dissolution of Sericin from Nonwoven into Wetting Fluid



Treated Fiber

Fluid Front Containing Fluorescent Sericin

FIG. 4

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Skin Coated with Sericin

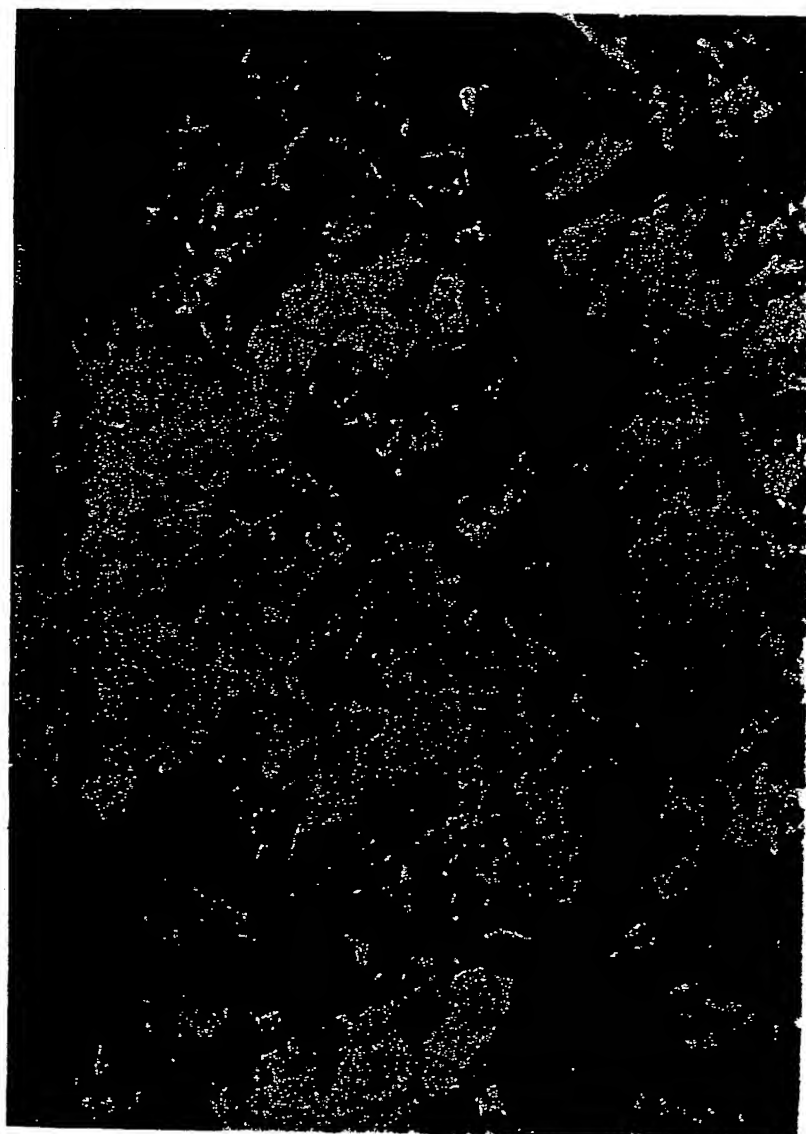


FIG. 5

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Dry Transfer of Sericin to Skin
(a) Brightfield and (b) Fluorescent Image, both 100x



Localized Deposition of Sericin

FIG. 6

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Inhibition of Fecal Proteolytic Activity with Aqueous
Zinc Salt

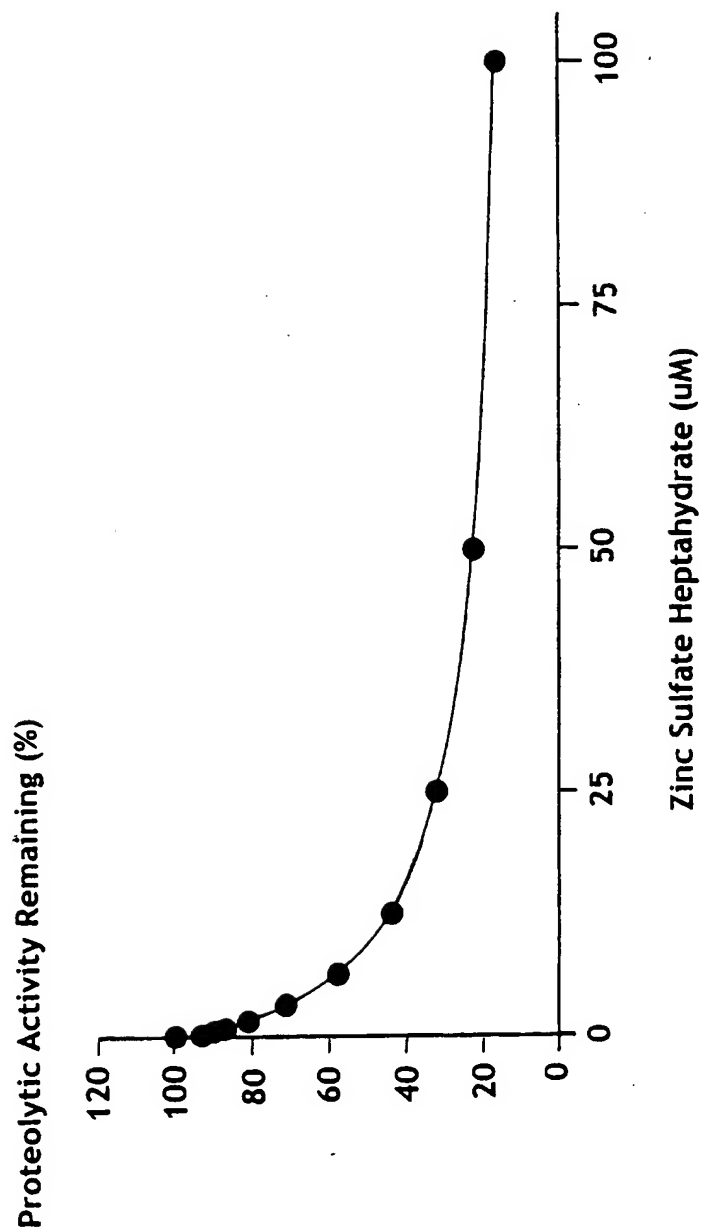


FIG. 7

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Inhibition of Proteolytic Activity in Infant Urine by Zinc Salt

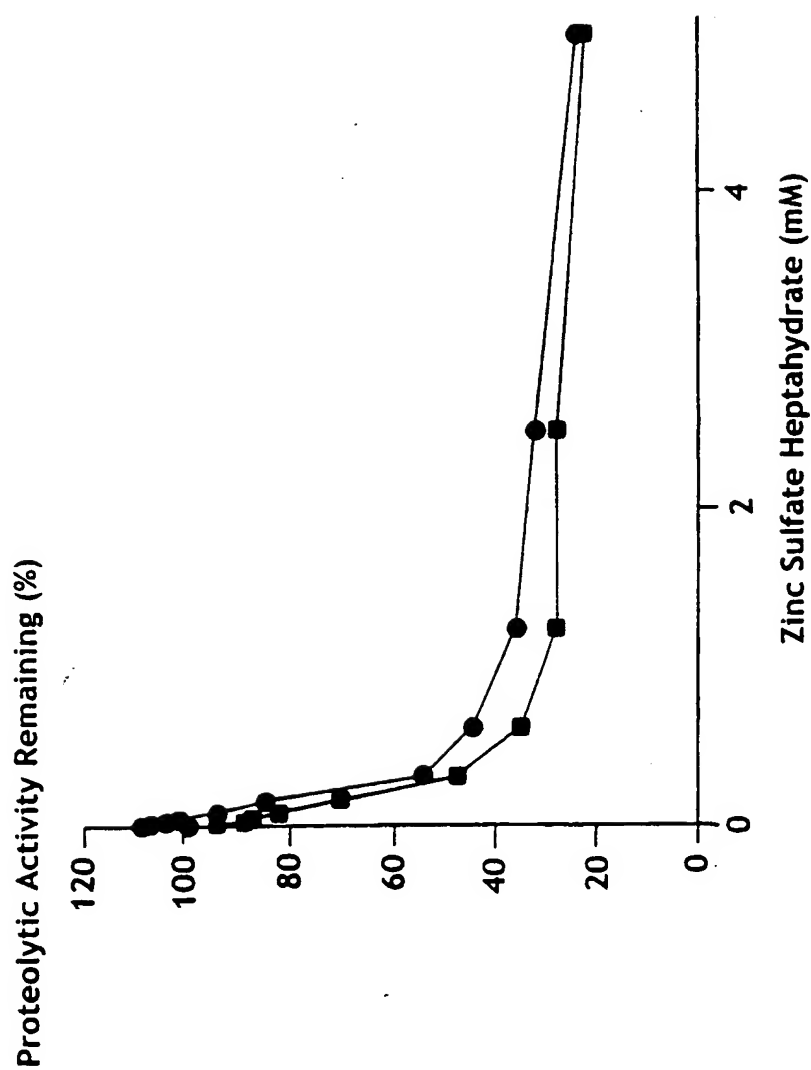


FIG. 8

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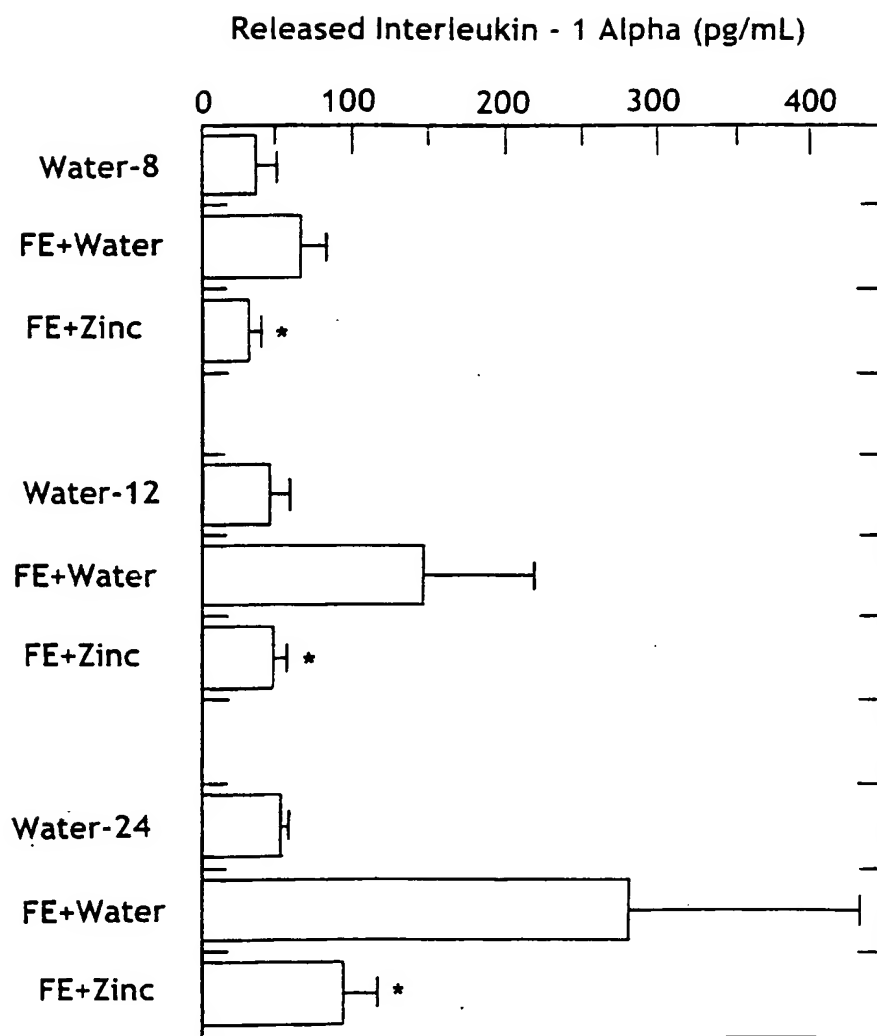
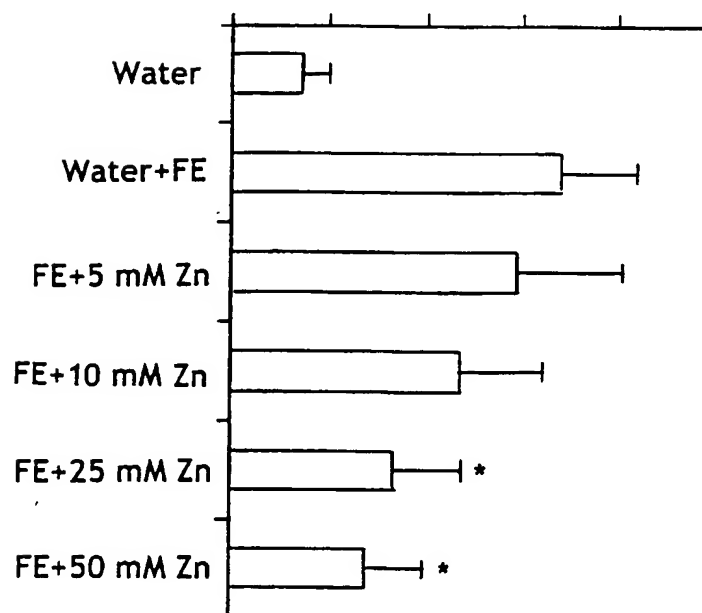


FIG. 9

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**Zinc Salt Reduces Fecal Extract
(FE)-induced Pro-inflammatory
Response in Human Skin Model**

Interleukin-1 Alpha Released (pg/mL)



Asterisk: statistical, student t-test (95% confidence interval)

FIG. 10

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 00/17999

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K7/48 A61K7/50 A61L15/48 A61L15/18 A61L15/32		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K A61L		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 875 233 A (FORT JAMES CORP) 4 November 1998 (1998-11-04) page 3, line 6 - line 10 page 3, line 14 - line 34 page 3, line 49 - page 4, line 2 page 7, line 17 - line 30 page 8, line 1 - line 2 page 9, line 41 - line 55 claims	1-29
Y	WO 96 16681 A (PROCTER & GAMBLE) 6 June 1996 (1996-06-06) page 3, line 32 - line 35 page 6, line 15 - line 25 page 26, line 17 - page 29, line 18 claims	1-29
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		
<input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Z" document member of the same patent family		
Date of the actual completion of the international search 4 October 2000		Date of mailing of the international search report 13/10/2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Cousins-Van Steen, G

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 00/17999

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 84 02845 A (ADVANCED DRUG TECH) 2 August 1984 (1984-08-02) page 2, line 9 - line 16 page 4, line 24 - line 29 page 6, line 20 -page 7, line 12 ---	1-5, 8-14, 17-28
Y	WO 91 02538 A (MORRIS JOHN CO INC) 7 March 1991 (1991-03-07) page 5, paragraph 1 - paragraph 2 page 12, paragraph 4 -page 15, paragraph 1 page 16, paragraph 2 -page 19, paragraph 2 claims ---	1-6
Y	EP 0 761 867 A (SEIREN CO LTD) 12 March 1997 (1997-03-12) page 2, line 24 - line 29 page 2, line 49 -page 3, line 15 claims ---	1,6,7, 15,16, 24,29
A	US 5 091 193 A (NOEL HUGUES ET AL) 25 February 1992 (1992-02-25) ---	
A	US 4 973 473 A (FERONE JAMES J ET AL) 27 November 1990 (1990-11-27) ---	
A	EP 0 750 903 A (COOPERATIE COSUN U A) 2 January 1997 (1997-01-02) -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/17999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0875233 A	04-11-1998	US 5871763 A	16-02-1999
WO 9616681 A	06-06-1996	US 5635191 A	03-06-1997
		AU 4135396 A	19-06-1996
		BR 9509775 A	04-11-1995
		CZ 9701612 A	12-11-1997
		EP 0794803 A	17-09-1997
		FI 972236 A	27-05-1997
		HU 77660 A	28-07-1998
		JP 10509895 T	29-09-1998
		NO 972395 A	28-07-1997
		TR 960503 A	21-07-1996
		ZA 9510060 A	02-07-1996
WO 8402845 A	02-08-1984	CA 1243951 A	01-11-1988
		EP 0133437 A	27-02-1985
WO 9102538 A	07-03-1991	AU 6425590 A	03-04-1991
		CA 2065044 A	19-02-1991
		EP 0487648 A	03-06-1992
		JP 5503071 T	27-05-1993
EP 0761867 A	12-03-1997	JP 9031847 A	04-02-1997
		US 5728461 A	17-03-1998
US 5091193 A	25-02-1992	FR 2651125 A	01-03-1991
		CA 2023810 A	24-02-1991
		DE 69001730 D	01-07-1993
		DE 69001730 T	04-11-1993
		EP 0414605 A	27-02-1991
		JP 3169822 A	23-07-1991
US 4973473 A	27-11-1990	JP 5504757 T	22-07-1993
		WO 9100083 A	10-01-1991
EP 0750903 A	02-01-1997	NL 1000681 C	31-12-1996

